IAEDANS   **AMPS**

5' AEDANS-S-AMP

FIGURE 3

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Inventor: Michelle M. Hanna
Tel. No.: 202-371-2600
For: Molecular Detection Systems Utilizing Reiterative
    Oligonucleotide Synthesis

FIGURE 13

Deaminated Methylated DNA

Deaminated Unmethylated DNA

$n R_1 - C\text{-}OH\ 3'$ Initiator
+
$n\ pppG - R_2$ Terminator

RNA Polymerase

$n\ R1 - CpG - R2$

Dinucleotide Product

Multiple Signals

NO PRODUCT
NO SIGNAL

FIGURE 14

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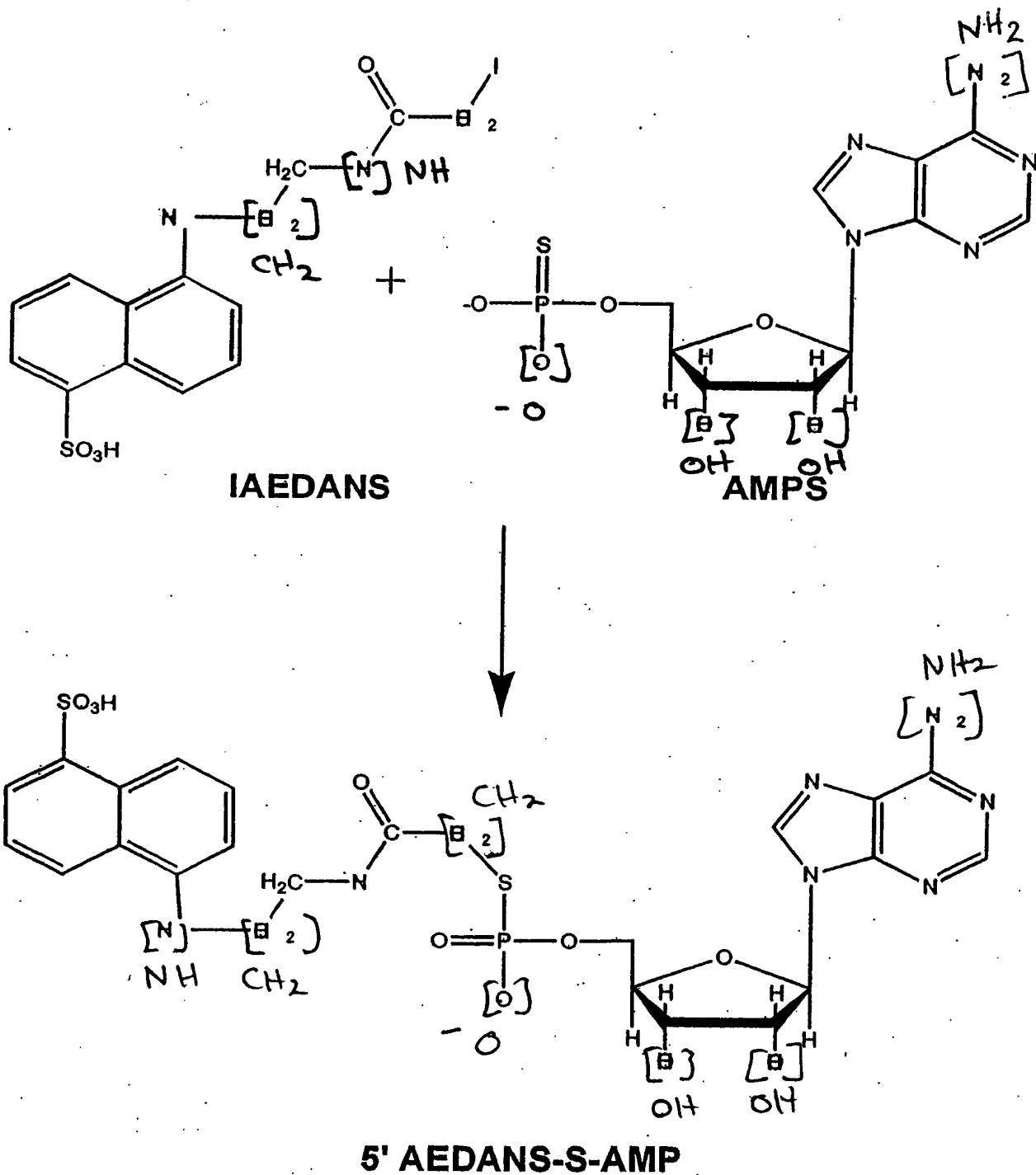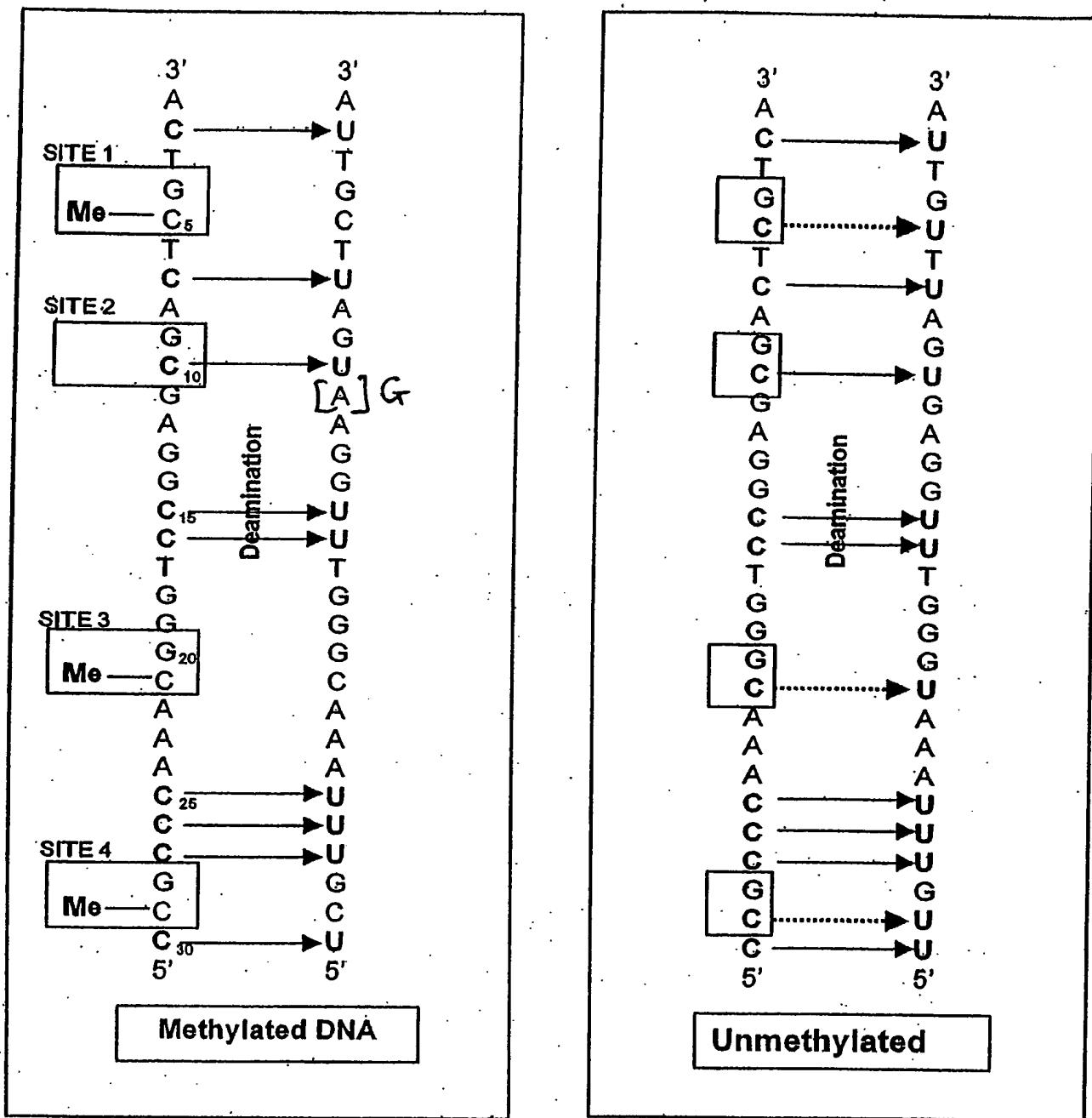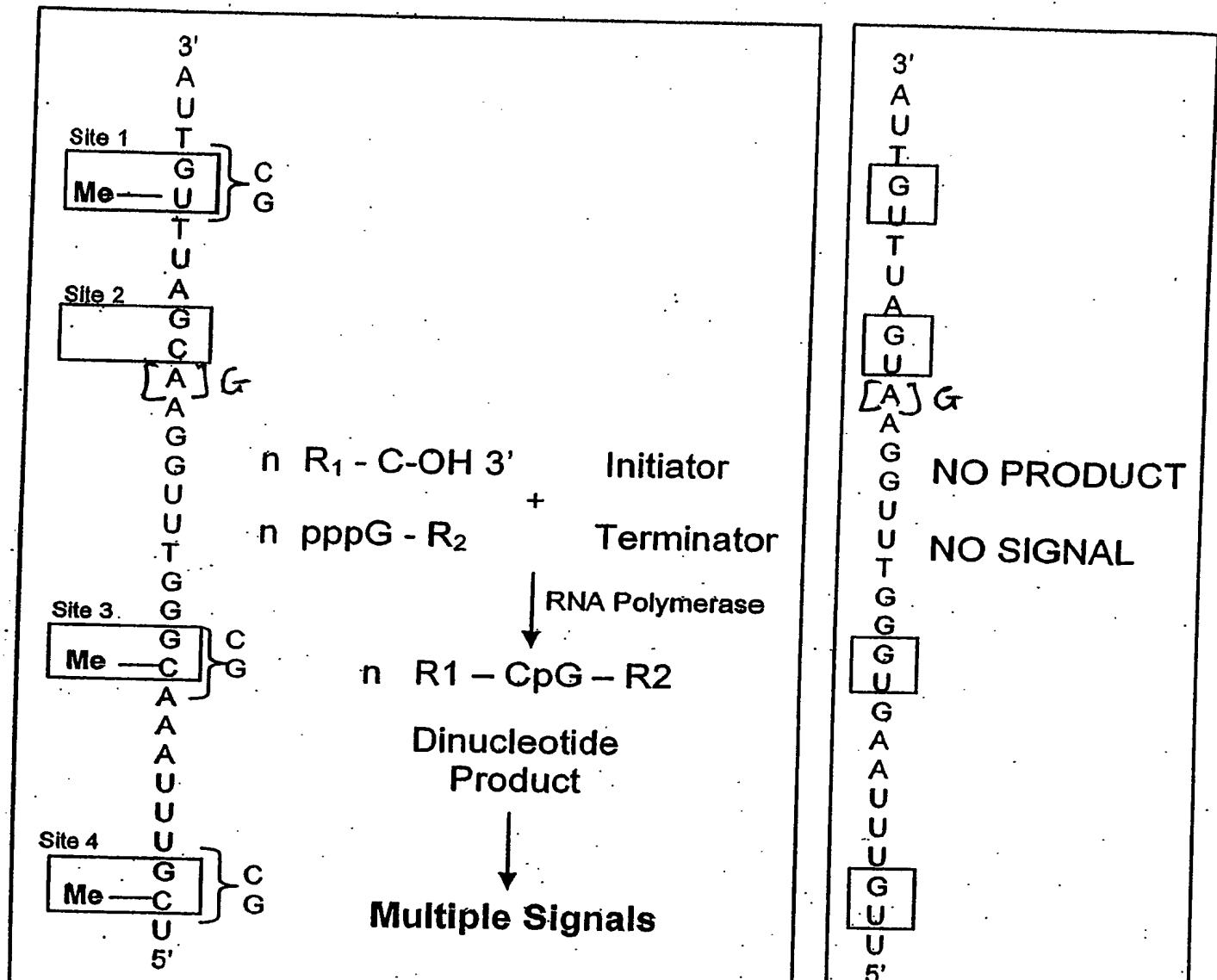
BEST AVAILABLE COPY

entire strand shifted down by 1 n.t., so that bases pair correctly.

$5'$ $R_1 - N_X - pC - OH$

$+ pppG - R_{2G}$ $+ pppA - R_{2A}$

$R_1N_X - CpG - R_2$ $R_1N_XCpA - R_{2A}$

λ_{2GE} λ_{2AE}

If C is Methylated   **If C is Not Methylated**
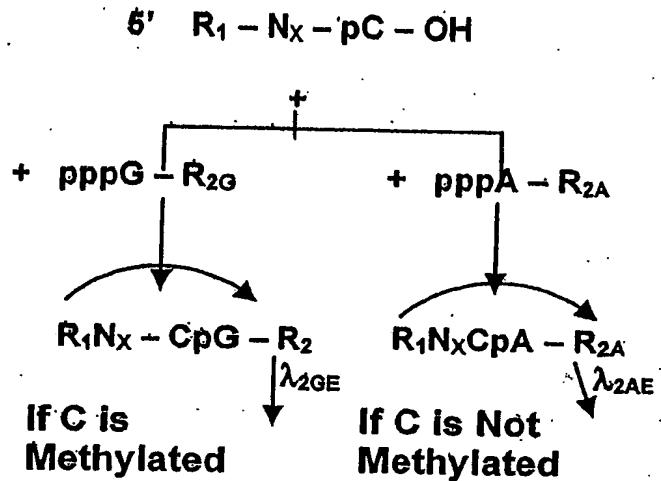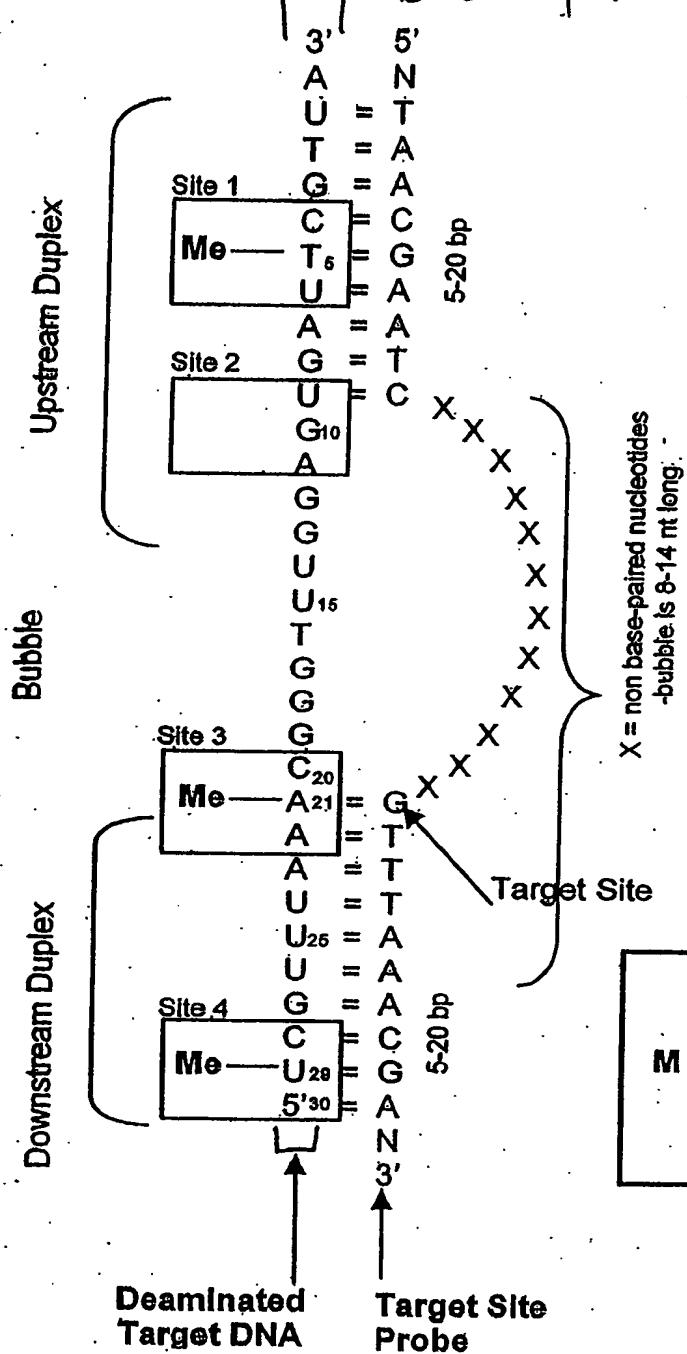
$M = 1$ If both copies are 100% methylated: Only λ_{2GE} detected

$M = 0.5$ If 1 copy is methylated: Both λ_{2GE} and λ_{2AE} detected

$M = 0$ If both copies unmethylated: only λ_{2AE} detected

$$M = \text{Methylation Index} = \frac{E\lambda_{2GE}}{E\lambda_{2GE} + E\lambda_{2GA}}$$

Upstream Duplex

Bubble

Downstream Duplex

$3'$ $5'$
A — N
U = T
T = A
G = A   Site 1
C — A
T_6 — C
U — G
A = A
G = T   Site 2
U — C
G_{10}
A
G
G
U
U_{15}
T
G
G
G   Site 3
C_{20}
A_{21} = G
A = T
A = T
U = T
U_{25} = A
U = A
G = A   Site 4
C = C
U_{29} = G
$5'_{30}$ = A
N
$3'$

Me (Site 1)
Me (Site 3)
Me (Site 4)

5-20 bp
5-20 bp

X = non base-paired nucleotides -bubble is 8-14 nt long.

X X X X X X X X X X X X X

Target Site

Deaminated Target DNA

Target Site Probe

FIGURE 15

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ATTATCCAGT
AGGCAGATTAAGCATGTGCTTAAGGCATCAGCAAAGTCTGAGCAATCCATTTTTTAAAACGTAGTACATGTTTT
TGATAAGCTTAAAAAGTAGTAGTCACAGGAAAAATTAGAACTTTTACCTCCTTGCGCTTGTTATACTCTTTAGT
GCTGTTTAACTTTTCTTTGTAAGTGAGGGTGGTGGAGGGTGCCCATAATCTTTTCAGGGAGTAAGTTCTTCTT
GGTCTTTCTTTCTTTCTTTCTTTCTTTTTTTCTTGAGACCAAGTTTCGCTCTTGTCTCCCAGGCTGGAGTGCAA
TGGCGCGATCTCGGCTCACTGCAACCTCCGCCTTCTCCTGGGTTCAAGCGATTCTCCTACATCAGCCTCCGA
GTAGCTGGGATTACAGGCATGCGCCACCAAGCCCCGCTAATTTTGTATTTTTAGTAGAGACAGGGTTTCGC
CATGTTGGTCAGGCTTGTCTCGAACTCCTGGCCTCAGGTGATCCGCCTGTCTCGGCCTCCCAGAATGCTGG
GATTATAGACGTGAGCCACCGCATCCGGACTTTCCTTTTATGTAATAGTGATAATTCTATCCAAAGCATTTTT
TTTTTTTTTGAGTCGGAGTCTCATTCTGTCACCCAGGCTGGAGGGTGGTGGCGCGATCTCGGCTTACTGCAA
CCTCTGCCTCCCGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGAATTACACACGTGCGCCA
CCATGGCCAGCTAATTTTTGTATTTTTAGTAGAGACGGGGTGTCACCATTTTGGCCAAGCTGGCCTCGAACTC
CTGACCTCAGGTGATCTGCCCGCCTCGGCTTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACCGCGTCCT
GCTCCAAAGCATTTTCTTTCTATGCCTCAAAACAAGATTGCAAGCCAGTCCTCAAAGCGGATAATTCAAGAGC
TAACAGGTATTAGCTTAGGATGTGTGGCACTGTTCTTAAGGCTTATATGTATTAATACATCATTTAAACTCACA
ACAACCCCTATAAAGCAGGGGGCACTCATATTCCCTTCCCCCTTTATAATTACGAAAAATGCAAGGTATTTTC
AGTAGGAAAGAGAAATGTGAGAAGTGTGAAGGAGACAGGACAGTATTTGAAGCTGGTCTTTGGATCACTGTG
CAACTCTGCTTCTAGAACACTGAGCACTTTTTCTGGTCTAGGAATTATGACTTTGAGAATGGAGTCCGTCCTT
CCAATGACTCCCTCCCCATTTTCCTATCTGCCTACAGGCAGAATTCTCCCCCGTCCGTATTAAATAAACCTCA
TCTTTTCAGAGTCTGCTCTTATACCAGGCAATGTACACGTCTGAGAAACCCTTGCCCCAGACAGCCGTTTTAC
ACGCAGGAGGGGAAGGGGAGGGGAAGGAGAGAGCAGTCCGACTCTCCAAAAGGAATCCTTTGAACTAGGG
TTTCTGACTTAGTGAACCCCGCGCTCCTGAAAATCAAGGGTTGAGGGGGGTAGGGGGACACTTTCTAGTCGTA
CAGGTGATTTCGATTCTCGGTGGGGCTCTCACAACTAGGAAAGAATAGTTTTGCTTTTTCTTATGATTAAAAGA
AGAAGCCATACTTTCCCTATGACACCAAACACCCCGATTCAATTTGGCAGTTAGGAAGGTTGTATCGCGGAG
GAAGGAAACGGGGCGGGGGCGGATTTCTTTTTAACAGAGTGAACGCACTCAAACACGCCTTTGCTGGCAGG
CGGGGGAGCGCGGCTGGGAGCAGGGAGGCCGGAGGGCGGTGTGGGGGGCAGGTGGGGAGGAGCCCAGT
CCTCCTTCCTTGCCAACGCTGGCTCTGGCGAGGGCTGCTTCCGGCTGGTGCCCCCGGGGGAGACCCAACC
TGGGGCGACTTCAGGGGTGCCACATTCGCTAAGTGCTCGGAGTTAATAGCACCTCCTCCGAGCACTCGCTC
ACGGCGTCCCCTTGCCTGGAAAGATACCGCGGTCCCTCCAGAGGATTTGAGGGGACAGGGTCGGAGGGGGC
TCTTCCGCCAGCACCGGAGGAAGAAAGAGGAGGGGCTGGCTGGTCACCAGAGGGTGGGGCGGACCGCGT
GCGCTCGGCGGCTGCGGAGAGGGGGAGAGCAGGCAGCGGGCGGCGGGGAGCAGCATGGAGCCGGCGGC
GGGGAGCAGCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCGCGGCCCGGGGTCGGGTAGAGGAGGT
GCGGGCGCTGCTGGAGGCGGGGGCGCTGCCCAACGCACCGAATAGTTACGGTCGGAGGCGATCCAGGT
GGGTAGAGGGTCTGCAGCGGGAGCAGGGGATGGCGGGCGACTCTGGAGGACGAAGTTTGCAGGGGAATT
GGAATCAGGTAGCGCTTCGATTCTCCGGAAAAAGGGGAGGCTTCCTGGGGAGTTTTCAGAAGGGGTTTGTA
ATCACAGACCTCCTCCTGGCGACGCCCTGGGGGCTTGGGAAGCCAAGGAAGAGGAATGAGGAGCCACGCG
CGTACAGATCTCTCGAATGCTGAGAAGATCTGAAGGGGGGAACATATTTGTATTAGATGGAAGTATGCTCTTT
ATCAGATACAAAATTTACGAACGTTTGGGATAAAAAGGGAGTCTTAAAGAAATGTAAGATGTGCTGGGACTAC
TTAGCCTCCAATTCACAGATACCTGGATGGAGCTTATCTTTCTTACTAGGAGGGATTATCAGTGGAAATCTGT
GGTGTATGTTGGAATAAATATCGAATATAAATTTTGATCGAAATTATTCAGAAGCGGCCGGGCGCGGTGCCTC
ACGCCTTGTAATCCCTTCACTTTGGGAGATCAAGGCGGGGGGAATCACCTGAGGTCGGGAGTTCGAGACCA
GCCTGGCCAACAGGTGAAACCTCGCCTCTACTAAAAATACAAAAAGTAGCCGGGGGGTGGTGGCAGGCGCCT
GTAATCCCAGCTACTCGGGAGGTTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCTGAGGTTGTAGTGAAC
AGCGAGATGGAGCCACTTCACTCCAGCCTGGGTGACAGAGTGAGACTTTGTCGAAAGAAAGAAAGAGAGAA
AGAGAGAGAGAAAAATTATTCAGAAGCAACTACATATTGTGTTTATTTTTAACTGAGTAGGGCAAATAAATATA
TGTTTGCTGTAGGAACTTAGGAAATAATGAGCCACATTCATGTGATCATTCCAGAGGTAATATGTAGTTACCAT
TTTGGGAATATCTGCTAACATTTTTGCTCTTTTACTATCTTTAGCTTACTTGATATAGTTTATTTGTGATAAGAG
TTTTCAATTCCTCATTTTTGAACAGAGGTGTTTCTCCTCTCCCTACTCCTGTTTTGTGAGGGAGTTAGGGGAG
GATTTAAAAGTAATTAATACATGGGTAACTTAGCATCTCTAAAATTTTGCCAACAGCTTGAACCCGGGAGTTTG
GCTTTGTAGTCCTACAATATCTTAGAAGAGACCTTATTTGTTTAAAAACAAAAAGGAAAAAGAAAAGTGGATAG
TTTTGACAATTTTTTAATGGAG

Figure [30]

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**1. Target capture
from patient DNA**

CGATCACGT

CH$_3$ CH$_3$
CGATCACGT

**2. C-Deamination
(+/- PCR)**

UGATUAUGT

CH$_3$ CH$_3$
CGATUACGT

Not methylated

Methylated

No Signal

Many Signals

FIGURE 31

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